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Variation of glucosinolates in vegetable crops of *Brassica rapa*

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Abstract

Glucosinolate levels in leaves were determined in a collection of 113 varieties of turnip greens (*Brassica rapa* L.) from northwestern Spain grown at two sites. Sensorial attributes were also assessed by a consumer panel. The objectives were to determine the diversity among varieties in total glucosinolate content and glucosinolate profile and to evaluate their sensory attributes in relation to glucosinolate content for breeding purposes. Sixteen glucosinolates were identified, being the aliphatic glucosinolates, gluconapin and glucobrassicanapin the most abundant. Other aliphatic glucosinolates, such as progoitrin, glucoalyssin, and gluconapoleiferin were relatively abundant in varieties with a different glucosinolate profile. Indolic and aromatic glucosinolate concentrations were low and showed few differences among varieties. Differences in total glucosinolate content, glucosinolate profile and bitterness were found among varieties, with a total glucosinolate content ranging from 11.8 to 74.0 μmol g^{-1} dw at one site and from 7.5 to 56.9 μmol g^{-1} dw at the other site. Sensory analysis comparing bitterness with variation in glucosinolate, gluconapin and glucobrassicanapin concentrations suggested that these compounds and their breakdown products are not the only determinants of the characteristic flavour of this vegetable. Other phytochemicals are probably involved on the characteristic bitter flavour. The varieties **MBG-BRS0132**, **MBG-BRS0082**, **MBG-BRS0173**, and **MBG-BRS0184** could be good candidates for future breeding programs since they had high total glucosinolate content and good agronomic performance. The presence of glucoraphanin in some varieties should be studied more extensively, because this aliphatic glucosinolate is the precursor of sulforaphane, a potent anti-cancer isothiocyanate.
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Keywords: *Brassica rapa*; Bitterness; Consumer panel; Glucosinolates; Local varieties; Turnips

1. Introduction

In Galicia (northwestern Spain), *Brassica rapa* subsp. *rapa* L. includes turnip greens and turnip tops as vegetable products. Turnip greens are the young leaves harvested in the vegetative period, while turnip tops are the fructiferous stems with the flower buds and the surrounding leaves. Both are boiled and generally consumed as meat companions. They are characterized by a particular bitter and pungent taste, which differentiate them from other vegetables in the genus *Brassica*, such as cabbage, broccoli, and cauliflower. The bitterness has been related to the content of some glucosinolate degradation products (Chong et al., 1982; Fenwick et al., 1983; Carlson et al., 1987; van Doorn et al., 1998; Schonhof et al., 2004). Detailed reviews on glucosinolates in *Brassica* have been reported by several authors (Heaney and Fenwick, 1980; Fenwick et al., 1983; Rosa et al., 1997; Rosa, 1999; Mithen, 2001).

Glucosinolates can be grouped into three chemical classes, aliphatic, indolyl, and aromatic glucosinolates, according to whether their amino acid precursor is methionine, tryptophan or an aromatic amino acid (tyrosine or phenylalanine) (Rosa, 1999). The most important glucosinolates are methionine-derived glucosinolates which are found in *Brassica* vegetables (Mithen et al., 2003). Glucosinolates are β-

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thioglucoside *N*-hydroxysulfates containing a side chain and a β-D-glucopyranose moiety. Upon cellular disruption glucosinolates are hydrolyzed to various bioactive breakdown products by the endogenous enzyme myrosinase (thioglucohydrolase; E.C. 3.2.1.147). These breakdown products include isothiocyanates, nitriles, thiocyanates, epithionitriles, and oxazolidines (Fenwick et al., 1983) depending on the substrate, pH conditions, availability of ferrous ions, and the level and activity of specific protein factors such as the epithiospecifier protein (ESP) (Halkier and Du, 1997).

Glucosinolates and their breakdown products are known to have important biological activity. The range of activities of these compounds is wide; some are beneficial while others are detrimental for human and animal consumption (Rosa et al., 1997). Progoitrin has been shown to be potentially goitrogenic in animals. However, there is no evidence for any goitrogenic effect on humans from *Brassica* consumption (Mithen, 2001). Some of these compounds have a chemoprotective effect related to a reduction in the risk of certain cancers in humans (Zhang and Talalay, 1994; Fahey et al., 2001; Mithen et al., 2003). In vitro and in vivo studies have reported that isothiocyanates affect many stages of cancer development including modulation of phase I and II detoxification enzymes (Zhang et al., 1992; Mithen et al., 2003). Sulforaphane, an isothiocyanate derived from glucoraphanin and present at high levels in broccoli, inhibited chemically-induced breast cancer in rats (Fahey et al., 1994) but other isothiocyanates derived from common brassica vegetables may as well exert comparable levels of biological activity (Mithen et al., 2003). Considerable research has been conducted on the nutritional value of isothiocyanates found in other crops, such as watercress (*Rorripa nasturtium aquaticum*), and cancer prevention. Phenethyl isothiocyanate (PEITC) is a derivate of the glucosinolate gluconasturtiin, which occurs in large quantities in watercress. Rose et al. (2005) showed that extracts of broccoli and watercress inhibit the invasive potential of the human breast cancer cell line in vitro and suggested that their phytochemical constituents, the isothiocyanates, are a new class of invasion inhibitors.

Most research on vegetable crops of the genus *Brassica* has been focussed on *Brassica oleracea* (Zhang et al., 1992; Nastruzzi et al., 1996; Cashman et al., 1999; Farnham et al., 2000; Farnham et al., 2004). In contrast, there is relatively little information in glucosinolate pattern in green tissues of *B. rapa*. No bioassay studies have been reported for these species but for Chinese cabbage (*B. rapa* subsp. *pekinenesis*) which intake has been inversely associated with the incidence of urothelial cancer (Sakauchi et al., 2005).

Isothiocyanates produce a pungent flavour and sulfurous aroma when the plant tissue is injured, playing a significant organoleptic role in *Brassica* products (Fenwick et al., 1983; Rosa, 1999). The direct relation between glucosinolate content and sensory properties has been studied in broccoli, cauliflower, and Brussels sprouts (Hansen et al., 1997; Baik et al., 2003; Schonhof et al., 2004), which differ from turnip greens and turnip tops in their glucosinolate composition. Those crops also have a significant sugar content, which decreases their bitter taste (Rosa et al., 2001; Schonhof et al., 2004). A sensory profile of turnip greens as affected by variety and maturity has been reported by Jones and Sanders (2002), which concluded that both sources had a significant effect on their sensory characteristics and preferences.

At the Misión Biológica de Galicia (Spanish Council for Scientific Research), a collection of *B. rapa* subsp. *rapa* varieties is maintained as part of the *Brassica* genus germplasm bank. Padilla et al. (2005) evaluated and classified this collection, based on morphological and agronomical traits, but no information is available about its nutritional and organoleptic properties, except for a study on the glucosinolate and fatty acid profiles in seeds of 12 varieties (De Haro et al., 1995). With the increased interest in diet and health it is necessary to have information of profiles and levels of glucosinolates in this *Brassica* species. This information should be useful for developing new cultivars with an appropriate glucosinolate profile, from which high quality added value products can be produced. The most promising varieties for future breeding purposes would be those with the highest total glucosinolate content and, particularly, of glucosinolates with beneficial effects related to human health.

The objectives of this study were: (i) to determine the diversity of total glucosinolate content and profiles in leaves of *B. rapa* varieties from northwestern Spain (ii) to evaluate sensory attributes of *B. rapa* varieties in relation to glucosinolate content for breeding purposes.

2. Results and discussion

Sixteen glucosinolates, belonging to the three chemical classes, were detected in the whole collection (Table 1). Three glucosinolates were detected in all varieties: glucobrassicanapin, glucobrassicin, and gluconasturtiin and four more were detected in about 90% of the varieties: gluconapin, progoitrin, glucoiberin, and neoglucobrassicin. Varieties showed significant differences in both sites for total glucosinolate content and for the seven glucosinolates mentioned above (Table 2). Epiprogoitrin, glucoraphanin, glucoerucin, and 4-methoxyglucobrassicin were present in a few varieties (Table 1) and no statistical differences in concentrations among varieties were found (Table 2). For gluconapoleiferin, glucoalyssin, 4-hydroxyglucobrassicin, and glucoiberverin, analyses of variance showed significant differences among varieties (Table 2).

The combined analysis of variance showed significant differences for total glucosinolate content among sites, varieties, and the site × variety interaction (Table 2). Considering the chemical classes, previous studies suggest that indolyl glucosinolates are more sensitive to environmental effects and less to the genotype than aliphatic glucosinolates (Kushad et al., 1999; Brown et al., 2002; Kim et al.,

Table 1
Glucosinolates identified in 113 varieties of *Brassica rapa*, their trivial name, abbreviations and varieties with the glucosinolate (%)

Chemical class systematic name	Trivial name	Abbreviation	Percentage of varieties
Aliphatic			
3-Butenyl	Gluconapin	GNA	98.2
4-Pentenyl	Glucobrassicanapin	GBN	100.0
2-(*R*)-2-Hydroxy-3-butenyl	Progoitrin	PRO	97.3
2-(*S*)-2-Hydroxy-3-butenyl	Epiprogoitrin	E-PRO	17.7
3-Methylsulphinylpropyl	Glucoiberin	GIB	88.5
3-Methylthiopropyl	Glucoiberverin	GIV	76.1
4-Methylsulphinylbutyl	Glucoraphanin	GRA	23.9
5-Methylsulphinylpentyl	Glucoalyssin	GAL	15.9
2-Hydroxy-4-pentenyl	Gluconapoleiferin	GNL	8.0
4-Methylthiobutyl	Glucoerucin	GER	2.7
Indolyl			
3-Indolylmethyl	Glucobrassicin	GBS	100.0
1-Methox-3-indolylmethyl	Neoglucobrassicin	NGBS	95.6
4-Hydrox-3-indolylmethyl	4-Hydroxyglucobrassicin	4-OHGBS	52.2
4-Methoxy-3-indolylmethyl	4-Methoxyglucobrassicin	4-OMGBS	2.7
Aromatic			
2-Phenylethyl	Gluconasturtiin	GST	100

Table 2
Mean squares of the analysis of variance within each site and mean squares of the combined analysis of variance for the glucosinolate content in the *Brassica rapa* varieties from northwestern Spain evaluated at two sites

Traits	Site 1		Site 2		Combined analysis		
	Variety	Error	Variety	Error	Site	Variety	Site × variety
Total glucosinolate	525.1**	113.8	301.3**	99.8	5515.0**	722.6**	201.2**
Aliphatic							
GNA	517.9**	116.7	350.1**	83.4	3382.6**	778.6**	211.7**
GBN	31.7**	10.8	29.2**	7.1	14.9	51.8**	11.7
PRO	31.6**	11.2	12.2**	5.2	19.5	39.7**	18.2**
E-PRO	0.05	0.06	0.006	0.005	–	–	–
GIB	3.87**	0.18	4.13**	0.20	0.01	7.48**	0.37**
GIV	0.21**	0.12	0.53**	0.14	0.02	0.36**	0.09
GRA	0.003	0.003	0.9	1.3	–	–	–
GAL	51.7**	13.6	93.1**	21.4	17.8	132.6**	18.5
GNL	3.02**	0.15	1.19**	0.08	0.04	5.09**	0.68**
Indolyl							
GBS	0.21**	0.07	0.04**	0.02	2.8**	0.13	0.11**
NGBS	0.25**	0.11	0.03**	0.02	5.8**	0.2	0.1**
4-OHGBS	0.027**	0.006	0.002**	0.001	0.21**	0.02	0.02**
Aromatic							
GST	2.68**	0.67	0.25**	0.09	50.8**	2.1**	1.0**
Degrees of freedom	94	364	80	230	1	62	62

GNA: gluconapin; GBN: glucobrassicanapin; PRO: progoitrin; E-PRO: epiprogoitrin; GIB: glucoiberin; GIV: Glucoiberverin; GRA: glucoraphanin; GAL: glucoalyssin; GNL: gluconapoleiferin; GBS: glucobrassicin; NGBS: neoglucobrassicin; 4-OHGBS: 4-hydroxyglucobrassicin; GST: gluconasturtiin.
** Significant at $P \leqslant 0.01$.

2003). For all three indolyl glucosinolates (4-hydroxiglucobrassicin, glucobrassicin, and neoglucobrassicin), the site and site × variety interaction significantly affected concentrations of these compounds while variety did not significantly affected concentrations (Table 2). There were significant differences among varieties for all aliphatic glucosinolates and for gluconasturtiin (an aromatic glucosinolate) in the combined analysis of variance (Table 2). Moreover, site and site × variety interaction significantly affected gluconapin concentration (Table 2). Aliphatic glucosinolates are synthesized from methionine and a small number of genes regulate side-chain elongation and side-chain modifications, giving different aliphatic glucosinolates (Giamoustaris and Mithen, 1996). Hydroxylation of the gluconapin side-chain produces progoitrin. The biosynthetic relationships between glucosinolates of interest are shown in Fig. 1. Brown et al. (2002); and Zhao et al. (1994) demonstrated that sulphur deficiency reduces the

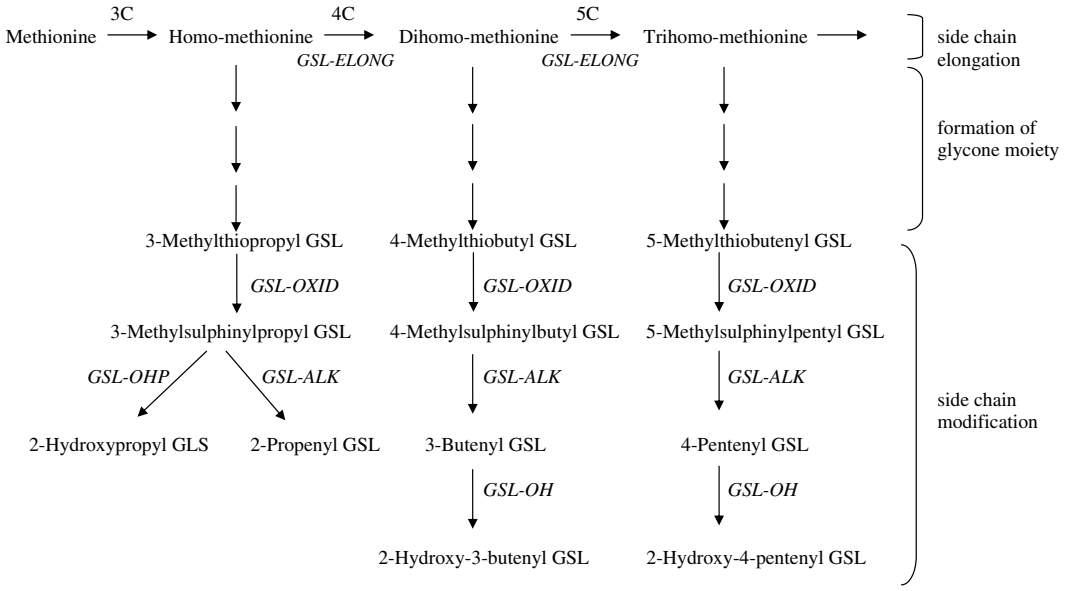

Fig. 1. A biochemical genetic model of the biosynthesis of aliphatic glucosinolates including the major genes controlling this process (Halkier and Du, 1997).

aliphatic glucosinolate concentration and high nitrogen supply favors the hydroxylation step. Brown et al. (2002), according to the results of Zhao et al. (1994), suggested that different environments (planting seasons) could inhibit the hydroxylation step that links gluconapin and progoitrin and observed that other aliphatic glucosinolates, such as glucoraphanin, were less affected by environmental stress. In this study, we found that glucobrassicanapin was less affected by environmental factors than other glucosinolates since neither site nor site × variety interaction showed significant differences for glucobrassicanapin (Table 2).

The total glucosinolate content ranged from 11.8 to 74.0 μmol g^{-1} dw in site 1 and from 7.5 to 56.9 μmol g^{-1} dw in site 2, with mean values of 34.5 and 26.0 μmol g^{-1} dw, in site 1 and 2, respectively (Table 3). These contents are lower than those found in other *B. rapa* varieties (Kim et al., 2003). The average glucosinolate content detected in leaves collected from site 1 was higher than those collected from site 2 (Table 3). Variation on the amount and pattern of glucosinolates in *Brassica* plants has been attributed to genetic and environmental factors, including plant age, temperature, water stress, and soil type (Rosa et al., 1997). Soil differences across sites may be the cause of the significant differences between sites and site × variety interaction for gluconapin (Table 2), as the response to different edaphic conditions could vary from certain genotypes to others. In fact, soil analyses indicated that site 2 was extremely acidic. Besides the influence of environment, the difference in glucosinolate content between sites could also be due to plant size and development. In a previous study, the environment had significant effects on traits related to plant morphology and earliness in the same *B. rapa* populations used for this study (Padilla et al., 2005) which could also explain the differences in glucosinolate concentrations between the two sites.

Aliphatic glucosinolate were predominant, with gluconapin as the most common glucosinolate at both sites, followed by glucobrassicanapin (Table 3). Progoitrin, considered an anti-nutritional glucosinolate, was present in most varieties although the concentration was low (mean contents of 1.4 μmol g^{-1} dw in site 1 and 1.1 μmol g^{-1} dw in site 2). Indolyl and aromatic glucosinolate contents were low, being gluconasturtiin the most abundant (Table 3). Previous studies found the same glucosinolate pattern in leaves (Kim et al., 2003), flower buds (Carlson et al., 1987; Rosa, 1997; Kim et al., 2003), and seeds of *B. rapa* (De Haro et al., 1995) proving that in this species there is little variation in glucosinolate content.

Most varieties had a proportion of gluconapin between 70% and 95% of the total glucosinolate content and a proportion of glucobrassicanapin below 20% of the total glucosinolate content. The percentages of other minor glucosinolates, such as glucoiberin, progoitrin, glucoalyssin, and gluconasturtiin, were lower than 20% of the total glucosinolate content. This profile gives the standard pattern.

The hydrolysis products of gluconapin and glucobrassicanapin are isothiocyanates, nitriles, and epithionitriles. Isothiocyanates have beneficial effects on human health. Therefore, the most promising varieties for future breeding purposes would be those with the highest total glucosinolate content. The variety **MBG-BRS0132** had the highest total glucosinolate content (74.4 μmol g^{-1} dw in site 1 and 56.9 μmol g^{-1} dw in site 2) (Fig. 2a) and the highest gluconapin content (64.3 μmol g^{-1} dw in site 1 and 47.6 μmol g^{-1} dw in site 2). Other varieties with high total glucosinolate content at both sites were **MBG-BRS0082**, **MBG-BRS0184**, **MBG-BRS0259** and **MBG-BRS0261** (Fig. 2a). These varieties displayed the standard glucosinolate profile, except for **MBG-BRS0184** in site 1 which had

Table 3
Mean and range (μmol g^{-1} dw) for total glucosinolate content and individual glucosinolates for 63 varieties of *Brassica rapa* from northwestern Spain evaluated at two sites

	Site 1			Site 2		
	Mean	Range	LSD (5%)	Mean	Range	LSD (5%)
Total glucosinolate	34.5	11.8–74.0	17.0	26.0	7.5–56.9	15.9
Aliphatic						
GNA	25.5	0–64.3	17.2	18.3	0–47.6	14.6
GBN	2.6	0.2–14.6	5.2	2.4	0.2–15.4	4.3
PRO	1.4	0–17.2	5.3	1.1	0–7.2	3.7
E–PRO	0.03	0–0.85	–	0.01	0–0.23	–
GIB	1.59	0–2.79	0.68	1.38	0–3.45	0.71
GIV	0.22	0–1.07	0.56	0.24	0–2.38	0.58
GRA	0.01	0–0.04	–	≈0	0–0.08	–
GAL	1.2	0–27.0	5.9	1.6	0–25.5	7.4
GNL	0.11	0–7.48	0.60	0.12	0–3.76	0.45
GER	≈0	0–0.06	–	≈0	0–0.04	–
Indolyl						
GBS	0.34	0–1.07	0.42	0.18	0.04–0.48	0.24
NGBS	0.30	0–1.19	0.52	0.08	0–0.45	0.20
4-OHGB	0.05	0–0.54	0.13	0.01	0–0.20	0.05
4-OMGBS	≈0	0–0.02	–	0	0–0	–
Aromatic						
GST	1.08	0–3.80	1.31	0.38	0–1.64	0.49

GNA: gluconapin; GBN: glucobrassicanapin; PRO: progoitrin; E-PRO: epiprogoitrin; GIB: glucoiberin; GIV: Glucoiberverin; GRA: glucoraphanin; GAL: glucoalyssin; GNL: gluconapoleiferin; GER, glucoerucin; GBS: glucobrassicin; NGBS: neoglucobrassicin; 4-OHGBS: 4-hydroxyglucobrassicin; 4-OMGBS: 4-methoxyglucobrassicin; GST: gluconasturtiin.

relatively moderate gluconapin levels (Fig. 2b). In a previous study, **MBG-BRS0132** displayed a good agronomic performance for turnip greens production (Padilla et al., 2005). Regarding both high glucosinolate content and the agronomic value of this crop, **MBG-BRS0082**, **MBG-BRS0173**, and **MBG-BRS0184** (Fig. 2a) have potential for developing genotypes with increased glucosinolate levels that have beneficial health effects. Two varieties, **MBG-BRS0102** (19.6 μmol g^{-1} and 7.6 μmol g^{-1} at sites 1 and 2, respectively) and **MBG-BRS0104** (24.0 μmol g^{-1} and 7.5 μmol g^{-1} at sites 1 and 2, respectively) had the lowest total glucosinolate content, at both sites (Fig. 2a). In this study, varieties with low total glucosinolate content have either higher or the same relative content of gluconapin than varieties with high total glucosinolate content (Fig. 2a and b).

Some varieties had atypical glucosinolate profiles (Fig. 3). **MBG-BRS0418** and **MBG-BRS0419** had relatively high levels of glucoalyssin [an intermediate glucosinolate in the glucobrassicanapin synthesis pathway (see Fig. 1)] and almost no gluconapin (close to 0 μmol g^{-1} dry weight) at both sites. The varieties **MBG-BR0S236** and **MBG-BRS0320** had low or moderate levels of gluconapin and high levels of progoitrin [a result of the hydroxylation of gluconapin (Fig. 1)], glucobrassicanapin, and gluconapoleiferin. These four varieties had atypical glucosinolate pattern at both sites, suggesting that their characteristics are genetically fixed and that they could have a modified or an interrupted glucosinolate biosynthesis pathway with respect to varieties with the standard glucosinolate profile. The varieties **MBG-BRS0401** and **MBG-BRS0413** had no gluconapin at all, but they were sampled only at one site. Of the five individual plants of each of these two varieties analyzed none had gluconapin. Finally, the glucosinolate pattern in the varieties **MBG-BRS0417** and **MBG-BRS0236** was different at each site (Fig. 3).

Regarding the sensory properties, there were significant differences among varieties in bitterness (Table 4). Seventeen varieties did not differ from the most bitter one (**MBG-BRS0075**) and there was no difference in bitterness between 31 varieties and the least bitter one (**MBG-BRS0345**). Glucosinolates were analyzed in 15 and 14 of the most bitter varieties, and 19 and 17 of the least bitter varieties from sites 1 and 2, respectively. The most bitter varieties had significant higher glucosinolate and gluconapin contents than the less bitter varieties at both sites and for glucobrassicanapin at site 1 (Table 5), suggesting a relationship between these two glucosinolates and bitterness. Several authors had reported the relation between the bitter taste and the content of some glucosinolate degradation products (Chong et al., 1982; Fenwick et al., 1983; Carlson et al., 1987; van Doorn et al., 1998; Schonhof et al., 2004). Rosa (1997) pointed out that the gluconapin was the responsible for the bitterness in turnip greens.

Thirty two varieties were analyzed at the two sites and evaluated by a consumer panel. The relation between bitterness and the total glucosinolate and relative gluconapin contents is shown in Fig. 4a and b, respectively. Total glucosinolate content appears to be related to bitter flavour but varieties with relatively high levels of gluconapin content

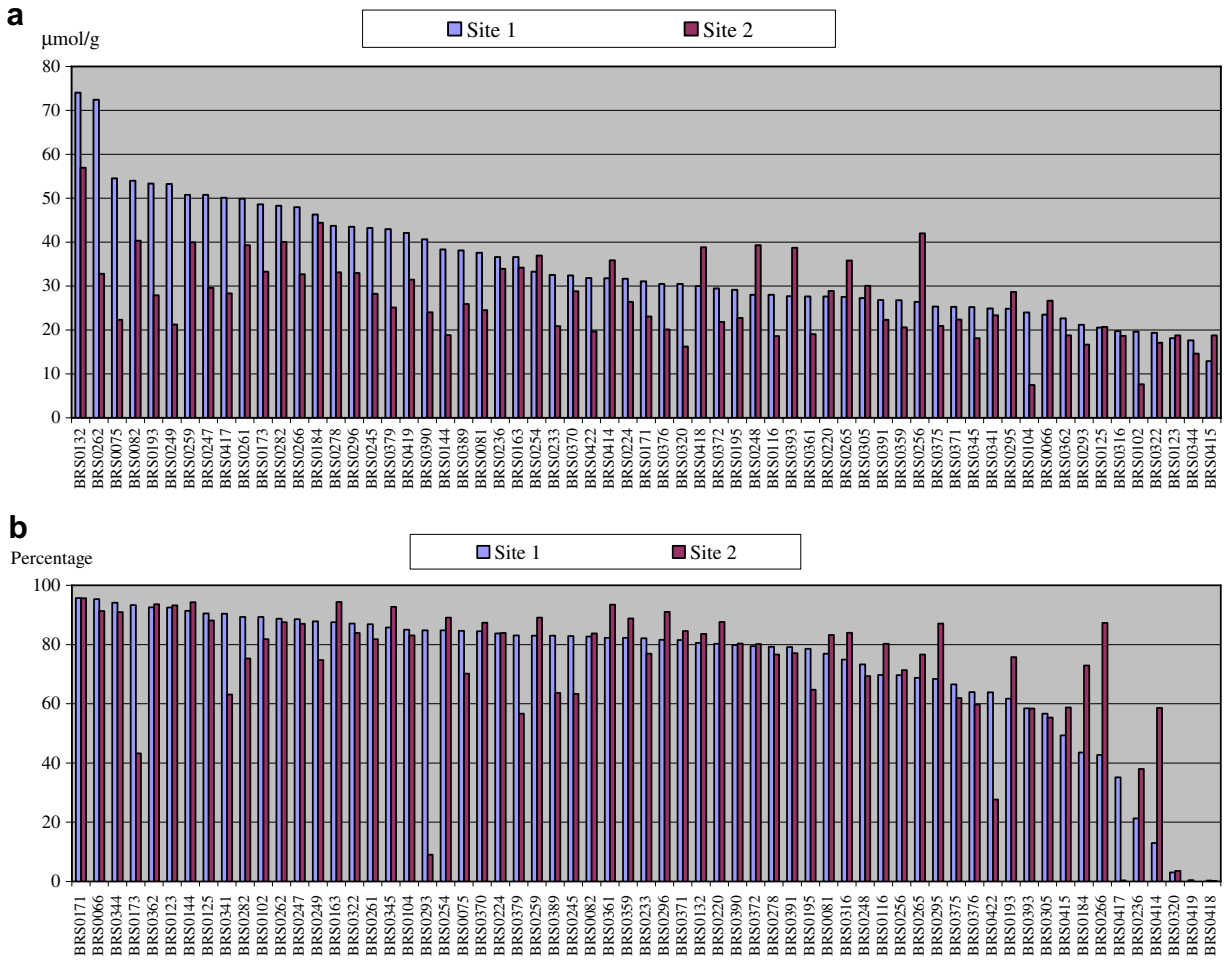

Fig. 2. (a) Total glucosinolate content expressed as (µmol/g) in the 63 varieties of *Brassica rapa* sampled at both sites. (b) Gluconapin relative content expressed as percentage of total glucosinolates in the 63 varieties of *Brassica rapa* sampled at both sites.

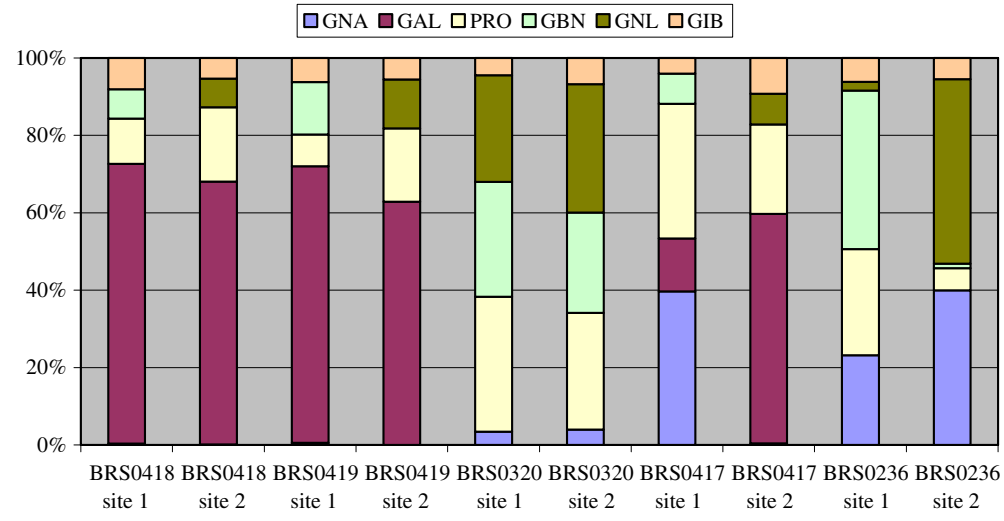

Fig. 3. Non-standard glucosinolate composition in five varieties of *Brassica rapa* sampled at two sites. GNA: gluconapin; GAL: glucoalyssin; PRO: progoitrin; GBN: glucobrassicanapin; GNL: gluconapoleiferin; GIB: glucoiberin; GRA: glucoraphanin.

were as bitter as varieties with low gluconapin content. Sensory analysis comparing bitterness with variation in glucosinolate concentrations suggests that these compounds and their breakdown products are not the only determinants of the characteristic flavour of this vegetable. Bitter taste is probably a synergistic property of various

Table 4
Mean squares for the sensory attributes in the 68 *Brassica rapa* varieties from northwestern Spain evaluated at two sites

Traits	Site	Variety	Site × variety
Bitterness	4.32**	0.48**	0.25
Flavour	2.60**	0.42	0.32
Degrees of freedom	1	67	67

Bitterness (rating scale from 1 = slight to 5 = high), flavour (rating scale from 1 = very bad to 5 = very good).
** Significant at $P \leqslant 0.01$.

phytochemicals, not just of hydrolysis products derived from gluconapin. Indole hydrolysis products, phenolic content, or flavonoids could have some influence on organoleptic properties and the bitter flavour of the varieties analyzed according to Drewnowski et al. (2001).

Variety **MBG-BRS0132**, with the highest glucosinolate and gluconapin contents did not differed from **MBG-BRS0075** for bitterness. The most bitter variety, **MBG-BRS0075**, had the third highest total glucosinolate content and gluconapin content at site 1, but it had moderate contents at site 2. The varieties **MBG-BRS0102** and **MBG-BRS0104**, with the lowest glucosinolate levels displayed a low bitterness (a value near to '2' using a rating scale from 1 to 5) (Fig. 4a). Three of the four varieties without gluconapin in one site, **MBG-BRS0401**, **MBG-BRS0413**, and **MBG-BRS0418** did not differed from the less bitter variety, but **MBG-BRS0419** was relatively bitter. This could be due to the total glucosinolate content of this variety, which was 31.5 and 42.1 μmol g^{-1}, values above mean values of all varieties at both sites, with glucoalyssin as its principal

Table 5
Mean values for total glucosinolate content and gluconapin and glucobrassicanapin contents for varieties of *Brassica rapa* with values extremes for bitterness evaluated at two sites

	Site 1			Site 2		
	Glucosinolate	GNA	GBN	Glucosinolate	GNA	GBN
More bitter varieties	42.8a	30.3a	3.08a	31.6a	23.1a	2.64a
Less bitter varieties	27.7b	19.6b	1.44b	23.6b	15.2b	2.54a

Values with different letter in the same column are significantly different ($P < 0.05$ Fisher's LSD test). GNA: gluconapin; GBN: glucobrassicanapin. Bitterness (rating scale from 1 = slight to 5 = high).

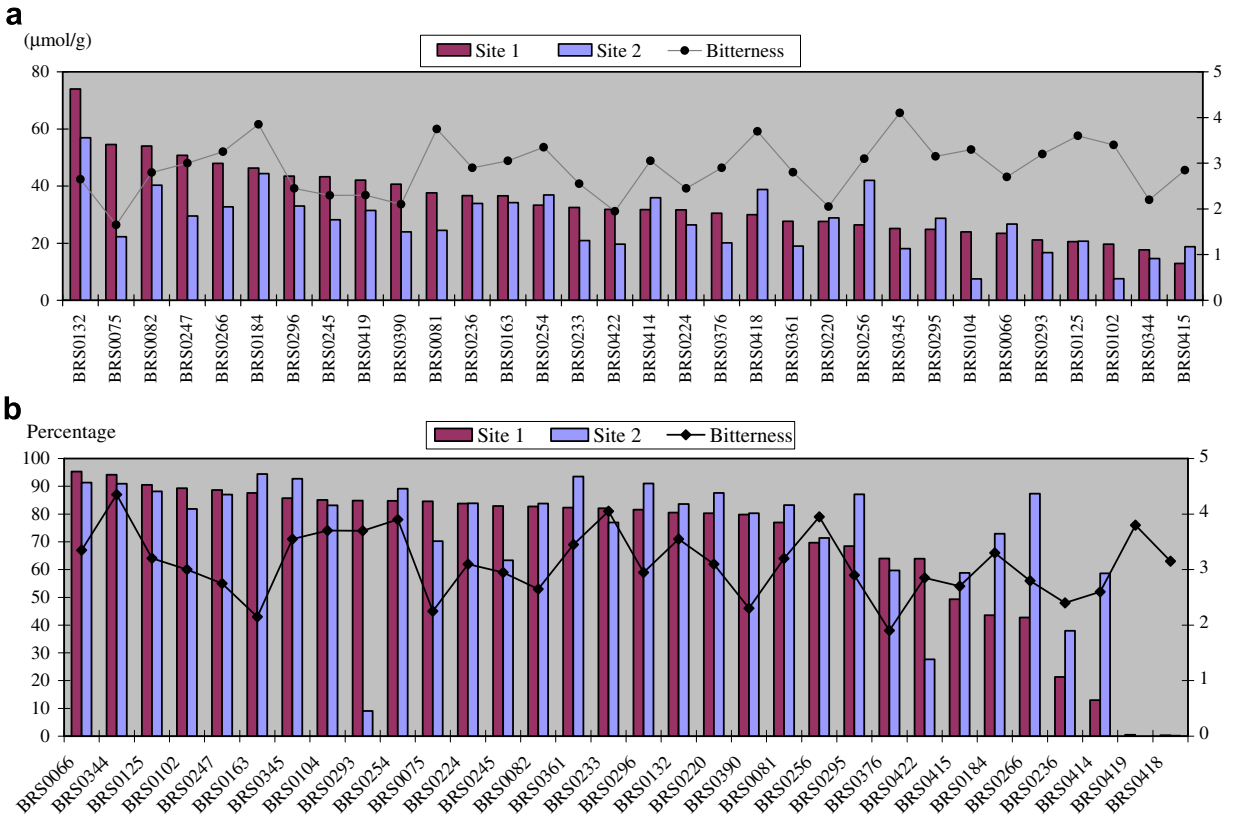

Fig. 4. (a) Bitterness expressed as rating scale from 1 = slight to 5 = high and total glucosinolate content expressed as (μmol/g) in 32 varieties of *Brassica rapa* sampled at both sites and evaluated by a consumer panel. (b) Bitterness and gluconapin relative content expressed as percentage of total glucosinolates in 32 varieties of *Brassica rapa* sampled at both sites and evaluated by a consumer panel.

glucosinolate. Therefore, this glucosinolate may be the responsible for the bitter impression in this variety. The relation between other glucosinolates, such as sinigrin and progoitrin with bitterness has been also reported by van Doorn et al. (1998) in Brussels sprouts.

Flavour attribute did not differ among varieties (Table 4). Flavour is probably very complex and difficult to evaluate objectively. However, the Spearman's coefficient of rank correlation between flavour and bitterness was $R = 0.76$, indicating that bitter-tasting varieties had a low acceptable taste, that indirectly relates the glucosinolate content with the flavour. However, a certain degree of bitterness is appreciated by consumers and desirable as it is a typical characteristic of this vegetable.

3. Conclusions

Varieties of *B. rapa* from northwestern Spain differed greatly in glucosinolate contents, glucosinolate profiles, and sensory properties. Qualitative differences observed among aliphatic composition may be due to allelic variation in a few genes encoding key regulatory enzymes at key points in the glucosinolate pathway. Our results suggest that the genes necessary for altering glucosinolate profiles can be found within *B. rapa* germplasm. Gluconapin was detected in most of the varieties and was the major glucosinolate. Glucoraphanin is the precursor of gluconapin in the glucosinolate biosynthesis pathway. Biosynthesis of gluconapin requires a functional allele at the *Gsl-alk* locus that converts glucoraphanin to its alkenyl homolog, gluconapin. Li and Quiros (2003) obtained transformed *Arabidopsis* plants with reduced concentration of glucoraphanin, which was converted into gluconapin. On the other hand, varieties with high glucoalyssin content enhance the possibility of studying the genes involved in glucosinolate regulation and the feasibility of modifying profiles in specific plant genotypes.

Consumers are aware of the need for a constant supply of phytochemical-containing plants for antioxidant support for diseases prevention. This study provides valuable information for future breeding programs as the ecological and nutritional effects of the glucosinolates are better known. Isothiocyanates derived from glucosinolates have a chemoprotective effect, related to a reduction in the risk of certain cancers in humans. Thus, **MBG-BRS0132**, **MBG-BRS0082**, **MBG-BRS0173**, and **MBG-BRS0184** could be good candidates for future breeding purposes because they had high total glucosinolate content and performed well agronomically in a previous study. In addition to isothiocyanate formation, the two main glucosinolates, gluconapin and glucobrassicanapin can be alternatively hydrolyzed to epithionitriles at the expense of corresponding isothiocyanates during glucosinolate hydrolysis in the presence of epithiospecifier protein and ferrous ions as they contains a terminal double bond in their side chain (Halkier and Gershenzon, 2006). While isothiocyanates have been demonstrated to have beneficial effects on human health, such information is lacking for epithionitriles.

The presence of glucoraphanin in 27 varieties should be studied more exhaustively since this aliphatic glucosinolate is the precursor of sulforaphane, a potent anti-cancer isothiocyanate. Bitterness was related to a high glucosinolate content, but not to a high gluconapin content, suggesting that other glucosinolates or other phytochemicals are involved in the characteristic bitter flavour of this *Brassica* species.

4. Experimental

4.1. Plant material

Leaves were harvested from the experimental fields described in Padilla et al. (2005) for glucosinolate analyses and the consumer panel test. The trials comprised 120 varieties from Galicia (northwestern Spain) and six commercial varieties and were performed at two locations. As plant material for glucosinolate analysis must be free of any kind of damage and leaves for the sensorial panel must be in good condition, it was not possible to analyze and consume all varieties evaluated in field trials. The glucosinolate analysis included 113 varieties, 63 of them sampled in both sites, 32 varieties sampled in Salcedo (site 1) and 18 varieties sampled in Forzanes (site 2). The consumer panel was done with 68 varieties, all of them sampled at both sites. Leaf harvest for both glucosinolate analysis and the consumer panel started two months after transplanting. The consumer panel evaluation lasted 25 days, as no more than 6 samples per day could be tasted.

4.2. HPLC analysis

A sample of healthy, fresh leaves was collected from three to five plants from each plot. The five upper leaves per plant (the two next to the apical leaf along with the adjacent three leaves) were sampled because they are the tender leaves used for human consumption. Leaf samples were frozen *in situ* and were taken immediately into the laboratory where they were stored at −80 °C. Then, the leaf samples were ground in liquid N_2, freeze-dried, and milled to a fine powder for the glucosinolate extractions. Glucosinolate composition was determined by HPLC according to Font et al. (2005). For each leaf sample, 100 mg dry wt was weighed and ground in a Janke and Kunkel, Model A10 mill (IKA-Labortechnik) for about 20 s and a two-step glucosinolate extraction was carried out in a water bath at 75 °C to inactivate myrosinase. In the first step, the sample was heated for 15 min in 2.5 ml 70% aqueous methanol and 200 μl 10 mM sinigrin (2-propenyl glucosinolate) as an internal standard. A second extraction was conducted after centrifugation (5 min, 5000*g*) by using 2 ml of 70% aqueous methanol. One milliliter of the combined glucosinolate extracts was pipetted onto the top of

an ion-exchange column containing 1 ml Sephadex DEAE-A25 in the formate form. Desulphation was carried out by the addition of 75 μl of purified sulphatase (E.C. 3.1.6.1, type H-1 from *Helix pomatia*) (Sigma) solution. Desulphated glucosinolates were eluted with 2.5 ml (0.5 ml × 5) Milli-Q (Millipore) ultra-pure water and analysed with a Model 600 HPLC instrument (Waters) equipped with a Model 486 UV tunable absorbance detector (Waters) at a wavelength of 229 nm. Separation was carried out by using a Lichrospher 100 RP-18 in Lichrocart 125-4 column, 5 μm particle size (Merck). HPLC solvents and gradient followed the ISO protocol (ISO Norm, 1992). The HPLC chromatogram was compared to the desulpho-glucosinolate profile of three certified reference materials recommended by U.E. and ISO (CRMs 366, 190 and 367) (Wathelet et al., 1991). The amount of each individual glucosinolate present in the sample was calculated with an internal standard, and expressed as μmol g^{-1} of dry wt. The total glucosinolate content was computed as the sum of all the individual glucosinolates present in the sample. Data were corrected for UV response factors for different types of glucosinolates (ISO Norm, 1992).

4.3. Consumer panel

The taste panel consisted of 12 members, regular consumers of turnip greens and turnip tops. Twenty five to 30 leaves from each plot were harvested according to the maturity cycle of each variety at the optimum time for consumption and boiled for 2 min in 1 dm^3 of water with 3 g salt. Samples were evaluated for bitterness and flavour on a continuous scale from '1' to '5'. For bitterness, a rating of 1 was considered 'slight' and 5 'high' and, for flavour, a rating 1 was 'very bad' and 5 'very good'. The sensory consumer test began after setting standards.

4.4. Statistical analyses

The experiment was set up in a completely random design and glucosinolate content per plant analyzed by individual analysis of variance. Varieties were considered as fixed effects. Comparisons of means among varieties were performed for each trait using the Fisher's protected least significant difference (LSD) at $p = 0.05$ (Steel et al., 1997). A combined analysis of variance across sites was carried out for varieties analyzed at both sites, in a randomized block design, with each site as a block and using the site × variety interaction as the experimental error. Varieties were considered as fixed effects and sites as random effects.

For sensory characteristics no sampling units were available, so an analysis of variance was carried out with the site × variety interaction as the error term, being varieties and sites fix and random effects, respectively. The Spearman's coefficient of rank correlation ($p < 0.05$) was calculated to correlate both bitterness and flavour sensory traits. A contrast analysis was performed to determine the relationship between glucosinolate content and bitterness and means were compared using the Fisher's LSD at $p = 0.05$ (Steel et al., 1997). All analyses were performed with the SAS statistical package (SAS Institute, 2000).

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